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(54) **Potato plant, tuben, seed and microtuber genetically engineered to form amylopectin-type  
starch**

(57) Potato plant, tuber, seed and microtuber having  
in the genome a gene construct comprising a fragment  
of the potato granule-bound starch synthase (GBSS)  
gene inserted in the antisense direction, the fragment  
being from SEQ ID No 1, 2 and 3 together with a pro-  
moter from the CaMV 35S, patatin I and GBSS promot-  
ers. Thereby suppressing amylose formation.

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## Description

The present invention relates to genetically engineered modification of potato, resulting in the formation of practically solely amylopectin-type starch in the potato. The genetically engineered modification implies the insertion of gene fragments into potato, said gene fragments comprising parts of leader sequence, translation start, translation end and trailer sequence as well as coding and noncoding (i.e. exons and introns) parts of the gene for granule-bound starch synthase, inserted in the antisense direction.

## Background of the Invention

Starch in various forms is of great import in the food and paper industry. In future, starch will also be a great potential for producing polymers which are degradable in nature, e.g. for use as packing material. Many different starch products are known which are produced by derivatisation of native starch originating from, inter alia, maize and potato. Starch from potato and maize, respectively, is competing in most market areas.

In the potato tuber, starch is the greatest part of the solid matter. About 1/4 to 1/5 of the starch in potato is amylose, while the remainder of the starch is amylopectin. These two components of the starch have different fields of application, and therefore the possibility of producing either pure amylose or pure amylopectin is most interesting. The two starch components can be produced from common starch, which requires a number of process steps and, consequently, is expensive and complicated.

It has now proved that by genetic engineering it is possible to modify potato so that the tubers merely produce mainly starch of one or the other type. As a result, a starch quality is obtained which can compete in the areas where potato starch is normally not used today. Starch from such potato which is modified in a genetically engineered manner has great potential as a food additive, since it has not been subjected to any chemical modification process.

## Starch Synthesis

The synthesis of starch and the regulation thereof are presently being studied with great interest, both on the level of basic research and for industrial application. Although much is known about the assistance of certain enzymes in the transformation of saccharose into starch, the biosynthesis of starch has not yet been elucidated. By making researches above all into maize, it has, however, been possible to elucidate part of the ways of synthesis and the enzymes participating in these reactions. The most important starch-synthesising enzymes for producing the starch granules are the starch synthase and the branching enzyme. In maize, three forms of starch synthase have so far been demonstrated and studied, two of which are soluble and one is insolubly associated with the starch granules. Also the branching enzyme consists of three forms which are probably coded by three different genes (Mac Donald & Preiss, 1985; Preiss, 1988).

## The Waxy Gene in Maize

The synthesis of the starch component amylose essentially occurs by the action of the starch synthase alpha-1,4-D-glucane-4-alpha-glucosyl transferase (EC 2.4.1.21) which is associated with the starch granules in the growth cell. The gene coding for this granule-bound enzyme is called "waxy" (=  $wx^+$ ), while the enzyme is called "GBSS" (granule-bound starch synthase).

waxy locus in maize has been thoroughly characterised both genetically and biochemically. The waxy gene on chromosome 9 controls the production of amylose in endosperm, pollen and the embryo sac. The starch formed in endosperm in normal maize with the  $wx^+$  allele consists to 25% of amylose and to 75% of amylopectin. A mutant form of maize has been found in which the endosperm contains a mutation located to the  $wx^+$  gene, and therefore no functioning GBSS is synthesised. Endosperm from this mutant maize therefore contains merely amylopectin as the starch component. This so-called waxy mutant thus contains neither GBSS nor amylose (Echt & Schwartz, 1981).

The GBSS protein is coded by the  $wx^+$  gene in the cell nucleus but is transported to and active in the amyloplast. The preprotein therefore consists of two components, viz. a 7 kD transit peptide which transfers the protein across the amyloplast membrane, and the actual protein which is 58 kD. The coding region of the  $wx^+$  gene in maize is 3.7 kb long and comprises 14 exons and 13 introns. A number of the regulation signals in the promoter region are known, and two different polyadenylating sequences have been described (Klößgen et al, 1986; Schwartz-Sommer et al, 1984; Shure et al, 1983).

## Amylose Enzyme in Potato

In potato, a 60 kD protein has been identified, which constitutes the main granule-bound protein. Since antibodies against this potato enzyme cross-react with GBSS from maize, it is assumed that it is the granule-bound synthase (Vos-

Scheperkeuter et al, 1986). The gene for potato GBSS has, however, so far not been characterised to the same extent as the waxy gene in maize, either in respect of locating or structure.

Naturally occurring waxy mutants have been described for barley, rice and sorghum besides maize. In potato no natural mutant has been found, but a mutant has been produced by X-radiation of leaves from a monohaploid ( $n=12$ ) plant (Visser et al, 1987). Starch isolated from tubers of this mutant contains neither the GBSS protein nor amylose. The mutant is conditioned by a simple recessive gene and is called amf. It may be compared to waxy mutants of other plant species since both the GBSS protein and amylose are lacking. The stability of the chromosome number, however, is weakened since this is quadrupled to the natural number ( $n=48$ ), which can give negative effects on the potato plants (Jacobsen et al, 1990).

#### Inhibition of Amylose Production

The synthesis of amylose can be drastically reduced by inhibition of the granule-bound starch synthase, GBSS, which catalyses the formation of amylose. This inhibition results in the starch mainly being amylopectin.

Inhibition of the formation of enzyme can be accomplished in several ways, e.g. by:

- mutagen treatment which results in a modification of the gene sequence coding for the formation of the enzyme
- incorporation of a transposon in the gene sequence coding for the enzyme
- genetically engineered modification so that the gene coding for the enzyme is not expressed, e.g. antisense gene inhibition.

Fig. 1 illustrates a specific suppression of normal gene expression in that a complementary antisense nucleotide is allowed to hybridise with mRNA for a target gene. The antisense nucleotide thus is antisense RNA which is transcribed in vivo from a "reversed" gene sequence (Izant, 1989).

By using the antisense technique, various gene functions in plants have been inhibited. The antisense construct for chalcone synthase, polygalacturonase and phosphotransferase has been used to inhibit the corresponding enzyme in the plant species petunia, tomato and tobacco.

#### Inhibition of Amylose in Potato

In potato, experiments have previously been made to inhibit the synthesis of the granule-bound starch synthase (GBSS protein) with an antisense construct corresponding to the gene coding for GBSS (this gene is hereinafter called the "GBSS gene"). Hergersberger (1988) describes a method by which a cDNA clone for the GBSS gene in potato has been isolated by means of a cDNA clone for the  $wx^+$  gene in maize. An antisense construct based on the entire cDNA clone was transferred to leaf discs of potato by means of *Agrobacterium tumefaciens*. In microtubers induced in vitro from regenerated potato sprouts, a varying and very weak reduction of the amylose content was observed and shown in a diagram. A complete characterisation of the GBSS gene is not provided.

Inhibition of the expression of the gene for granule-bound starch synthase in potato with heterologous (maize GBSS) antisense constructs has been described (Feenstra et al (1989), Abstract in the Handbook of the European Workshop on "Engineered Storage Products for the Agro Industry, 15-18/04/89, Bad Soden, Germany). The maximum reduction in amylose content of reserve starch attained with this method was 22%, i.e. a reduction from a total of 18% to 14%. Not all transformants with the same antisense construct showed the same response.

The gene for the GBSS protein in potato has been further characterised in that a genomic  $wx^+$  clone was examined by restriction analysis. However, the DNA sequence of the clone has not been determined (Visser et al, 1989).

Further experiments with an antisense construct corresponding to the GBSS gene in potato have been reported. The antisense construct which is based on a cDNA clone together with the CaMV 35S promoter has been transformed by means of *Agrobacterium rhizogenes*. According to information, the transformation resulted in a lower amylose content in the potato, but no values have been accounted for (Flavell, 1990).

None of the methods used so far for genetically engineered modification of potato has resulted in potato with practically no amylose-type starch.

The object of the invention therefore is to provide a practically complete suppression of the formation of amylose in potato tubers.

#### Summary of the Invention

According to the invention, the function of the GBSS gene and, thus, the amylose production in potato are inhibited by using completely new antisense constructs. For forming the antisense fragments according to the invention, the genomic GBSS gene is used as a basis in order to achieve an inhibition of GBSS and, consequently, of the amylose production, which is as effective as possible. The antisense constructs according to the invention comprise both coding

and noncoding parts of the GBSS gene which correspond to sequences in the region comprising promoter as well as leader sequence, translation start, translation end and trailer sequence in the antisense direction. For a tissue-specific expression, i.e. the amylose production should be inhibited in the potato tubers only, use is made of promoters which are specifically active in the potato tuber. As a result, the starch composition in other parts of the plant is not affected, which otherwise would give negative side-effects.

The invention thus comprises a fragment which essentially has one of the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 or SEQ ID No. 3. However, the sequences may deviate from those stated by one or more non-adjacent base pairs, without affecting the function of the fragments.

The invention also comprises a potato-tuber-specific promoter comprising 987 bp which belongs to the gene according to the invention, which codes for granule-bound starch synthase. Neither the promoter nor the corresponding gene has previously been characterised. The promoter sequence of 987 bp is stated in SEQ ID No. 4, while the gene sequence is stated in SEQ ID No. 5. Also the promoter and gene sequences may deviate from those stated by one or more non-adjacent base pairs, without affecting their function.

The invention also comprises vectors including the antisense fragments and the antisense constructs according to the invention.

In other aspects the invention comprises cells, plants, tubers, microtubers and seeds whose genome contains the fragments according to the invention inserted in the antisense direction.

In still further aspects, the invention comprises amylopectin-type starch, both native and derivatised.

Finally, the invention comprises a method of suppressing amylose formation in potato, whereby mainly amylopectin-type starch is formed in the potato.

The invention will now be described in more detail with reference to the accompanying Figures in which

Fig. 1 illustrates the principle of the antisense gene inhibition,  
 Fig. 2 shows the result of restriction analysis of the potato GBSS gene,  
 Fig. 3 shows two new binary vectors pHo3 and pHo4,  
 Fig. 4 shows the antisense constructs pHoxwA, pHoxwB and pHoxwD,  
 Fig. 5 shows the antisense constructs pHoxwF and pHoxwG, and  
 Fig. 6 shows the antisense constructs pHoxwK and pHoxwL.

Moreover, the sequences of the different DNA fragments according to the invention are shown in SEQ ID Nos 1, 2, 3, 4 and 5. There may be deviations from these sequences in one or more non-adjacent base pairs.

## MATERIALS

In the practical carrying out of the invention the following materials were used:

Bacterial strains: E. coli DH5alpha and DH5alpha'IQ(BRL). E. coli JM105 (Pharmacia). A. tumefaciens LBA4404 (Clontech).

Vectors: M13mp18 and mp19 (Pharmacia). pBI101 and pBI121 (Clontech). pBI240.7 (M. W. Bevan). pUC plasmids (Pharmacia).

Enzymes: Restriction enzymes and EcoRI linker (BRL). UNION™ DNA Ligation Kit (Clontech). Sequenase™ DNA Sequencing Kit (USB). T<sub>4</sub>-DNA ligase (Pharmacia).

The above-mentioned materials are used according to specifications stated by the manufacturers.

## Genomic Library

A genomic library in EMBL3 has been produced by Clontech on the applicant's account, while using leaves of the potato Bintje as starting material.

## Identification and Isolation of the GBSS Gene

The genomic library has been screened for the potato GBSS gene by means of cDNA clones for both the 5' and 3' end of the gene (said cDNA clones being obtained from M Hergersberger, Max Plank Institute in Cologne) according to a protocol from Clontech.

A full-length clone of the potato GBSS gene, wx311, has been identified and isolated from the genomic library. The start of the GBSS gene has been determined at an EcoRI fragment which is called fragment w (3.95 kb). The end of the GBSS gene has also been determined at an EcoRI fragment which is called fragment x (5.0 kb). A BglII-SpeI fragment which is called fragment m (3.9 kb) has also been isolated and shares sequences both from fragment w and from

fragment x. The fragments w, m and x have been subcloned in pUC13 (Viera, 1982; Yanisch-Peron et al, 1985) and are called pSw, pSm and pSx, respectively (Fig. 2).

#### Characterisation of the GBSS Gene in Potato

The GBSS gene in potato has been characterised by restriction analysis and cDNA probes, where the 5' and 3' end of the GBSS gene has been determined more accurately (Fig. 2). Sequence determination according to Sanger et al, 1977 of the GBSS gene has been made on subclones from pSw and pSx in M13mp18 and mp19 as well as pUC19 starting around the 5' end (see SEQ ID No. 5).

The promoter region has been determined at a BglII-NsiI fragment (see SEQ ID No. 4). Transcription and translation start has been determined at an overlapping BglII-HindIII fragment. The terminator region has in turn been determined at a SpeI-HindIII fragment.

#### Antisense Constructs for the GBSS Gene in Potato

The GBSS gene fragments according to the invention (see SEQ ID Nos 1, 2 and 3, and Fig. 2) have been determined in the following manner.

The restriction of pSw with NsiI and HindIII gives fragment I (SEQ ID No. 1) which subcloned in pUC19 is called 19NH35. Further restriction of 19NH35 with HpaI-SstI gives a fragment containing 342 bp of the GBSS gene according to the invention. This fragment comprises leader sequence, translation start and the first 125 bp of the coding region.

The restriction of pSm with HpaI and NsiI gives fragment II (SEQ ID No. 2) which subcloned in pJRD184 (Heusterspreute et al, 1987) is called pJRDmitt. Further restriction of pJRDmitt with HpaI-SstI gives a fragment containing 2549 bp of the GBSS gene according to the invention. This fragment comprises exons and introns from the middle of the gene.

The restriction of pSx with SstI and SpeI gives fragment III (SEQ ID No. 3) which subcloned in pBluescript (Melton et al, 1984) is called pBlue3'. Further restriction of pBlue3' with BamHI-SstI gives a fragment containing 492 bp of the GBSS gene according to the invention. This fragment comprises the last intron and exon, translation end and 278 bp of trailer sequence.

Antisense Constructs with Fragment I (Fig. 4): For the antisense construct pHoxwA, the HpaI-SstI fragment from 19NH35 has been inserted in the antisense direction into the binary vector pBI121 (Jefferson et al, 1987) cleaved with SmaI-SstI. The transcription of the antisense fragment is then initiated by the CaMV 35S promoter and is terminated by the NOS terminator (NOS = nopaline synthase).

For the antisense construct pHoxwB, the HpaI-SstI fragment from 19NH35 has been inserted in the antisense direction into the binary vector pHo4 (Fig. 3) cleaved with SmaI-SstI. The patatin I promoter which is tuber specific in potato comes from the vector pBI240.7 obtained from M. Bevan, Institute of Plant Science Research, Norwich. The transcription of the antisense fragment is then initiated by the patatin I promoter and is terminated by the NOS terminator.

For the antisense construct pHoxwD, the HpaI-SstI fragment from 19NH35 has been inserted in the antisense direction into the binary vector pHo3 (Fig. 3) cleaved with SmaI-SstI. pHo3 is a new binary vector which is constructed on the basis of pBI101. This vector which contains the promoter according to the invention (see SEQ ID No. 4) (GBSS promoter) of the now characterised potato GBSS gene according to the invention has been restrictioncleaved with SmaI and SstI, the HpaI-SstI fragment from 19NH35 being inserted in the antisense direction. The transcription of the antisense fragment is then initiated by its own GBSS promoter and is terminated by the NOS terminator. This means that the antisense fragment is transcribed only in the potato tuber, since the GBSS promoter like the patatin I promoter is tuber-specific.

Antisense Constructs with Fragment II (Fig. 5): For the antisense construct pHoxwF, the HpaI-SstI fragment from pJRDmitt has been inserted in the antisense direction into the binary vector pHo4 cleaved with SmaI-SstI. The transcription of the antisense fragment is then initiated by the patatin I promoter and terminated by the NOS terminator.

For the antisense construct pHoxwG, the HpaI-SstI fragment from pJRDmitt has been inserted in the antisense direction into the binary vector pHo3 cleaved with SmaI-SstI. The transcription of the antisense fragment is then initiated by its own GBSS promoter and is terminated by the NOS terminator.

Antisense Constructs with Fragment III (Fig. 6): For the antisense construct pHoxwK, the BamHI-SstI fragment from pBlue3' has been inserted in the antisense direction into the binary vector pHo4 cleaved with BamHI-SstI. The transcription of the antisense fragment is then initiated by the patatin I promoter and is terminated by the NOS terminator.

For the antisense construct pHoxwL, the BamHI-SstI fragment from pBlue3' has been inserted in the antisense direction into the binary vector pHo3 cleaved with BamHI-SstI. The transcription of the antisense fragment is then initiated

ated by its own GBSS promoter and is terminated by the NOS terminator.

The formed antisense constructs (Figs 4, 5, 6) have been transformed to *Agrobacterium tumefaciens* strain LBA4404 by direct transformation with the "freeze-thawing" method (Hoekema et al, 1983; An et al, 1988).

## 5 Transformation

The antisense constructs are transferred to bacteria, suitably by the "freeze-thawing" method (An et al, 1988). The transfer of the recombinant bacterium to potato tissue occurs by incubation of the potato tissue with the recombinant bacterium in a suitable medium after some sort of damage has been inflicted upon the potato tissue. During the incubation, T-DNA from the bacterium enters the DNA of the host plant. After the incubation, the bacteria are killed and the potato tissue is transferred to a solid medium for callus induction and is incubated for growth of callus.

After passing through further suitable media, sprouts are formed which are cut away from the potato tissue.

Checks for testing the expression of the antisense constructs and the transfer thereof to the potato genome are carried out by e.g. southern and northern hybridisation (Maniatis et al (1982)). The number of copies of the antisense construct which has been transferred is determined by southern hybridisation.

The testing of the expression on protein level is suitably carried out on microtubers induced in vitro on the transformed sprouts, thus permitting the testing to be performed as quickly as possible.

## Characterisation of the GBSS Protein

The effect of the antisense constructs on the function of the GBSS gene with respect to the activity of the GBSS protein is examined by extracting starch from the microtubers and analysing it regarding the presence of the GBSS protein. In electrophoresis on polyacrylamide gel (Hovenkamp-Hermelink et al, 1987), the GBSS protein forms a distinct band at 60 kD, when the GBSS gene functions. When the GBSS gene is not expressed, i.e. when the anti-sense GBSS gene is fully expressed, thereby inhibiting the formation of GBSS protein, no 60 kD band is demonstrated on the gel.

## Characterisation of the Starch

The composition of the starch in microtubers is identical with that of ordinary potato tubers, and therefore the effect of the antisense constructs on the amylose production is examined in microtubers. The proportion of amylose to amylopectin can be determined by a spectrophotometric method (e.g. according to Hovenkamp-Hermelink et al, 1988).

## Extraction of Amylopectin from Amylopectin Potato

Amylopectin is extracted from the so-called amylopectin potato (potato in which the formation of amylose has been suppressed by inserting the antisense constructs according to the invention) in a known manner.

## Derivatisation of Amylopectin

Depending on the final use of the amylopectin, its physical and chemical qualities can be modified by derivatisation. By derivatisation is here meant chemical, physical and enzymatic treatment and combinations thereof (modified starches).

The chemical derivatisation, i.e. chemical modification of the amylopectin, can be carried out in different ways, for example by oxidation, acid hydrolysis, dextrinisation, different forms of etherification, such as cationisation, hydroxypropylation and hydroxy ethylation, different forms of esterification, for example by vinyl acetate, acetic anhydride, or by monophosphatising, diphosphatising and octenyl succination, and combinations thereof.

Physical modification of the amylopectin can be effected by e.g. cylinder-drying or extrusion.

In enzymatic derivatisation, degradation (reduction of the viscosity) and chemical modification of the amylopectin are effected by means of existing enzymatic systems.

The derivatisation is effected at different temperatures, according to the desired end product. The ordinary range of temperature which is used is 20-45°C, but temperatures up to 180°C are possible.

The invention will be described in more detail in the following Examples.

## Example 1

Production of microtubers with inserted antisense constructs according to the invention

The antisense constructs (see Figs 4, 5 and 6) are transferred to *Agrobacterium tumefaciens* LBA 4404 by the "freeze-thawing" method (An et al, 1988). The transfer to potato tissue is carried out according to a modified protocol

from Rocha-Sosa et al (1989).

Leaf discs from potato plants cultured in vitro are incubated in darkness on a liquid MS-medium (Murashige & Skoog; 1962) with 3% saccharose and 0.5% MES together with 100 µl of a suspension of recombinant *Agrobacterium* per 10 ml medium for two days. After these two days the bacteria are killed. The leaf discs are transferred to a solid medium for callus induction and incubated for 4-6 weeks, depending on the growth of callus. The solid medium is composed as follows:

MS + 3% saccharose

2 mg/l	zeatin riboside
0.02 mg/l	"NAA"
0.02 mg/l	"GA <sub>3</sub> "
500 mg/l	"Claforan"
50 mg/l	kanamycin
0.25%	"Gellan"

Subsequently the leaf discs are transferred to a medium having a different composition of hormones, comprising:  
MS + 3% saccharose

5 mg/l	"NAA"
0.1 mg/l	"BAP"
500 mg/l	"Claforan"
50 mg/l	kanamycin
0.25%	"Gellan"

The leaf discs are stored on this medium for about 4 weeks, whereupon they are transferred to a medium in which the "Claforan" concentration has been reduced to 250 mg/l. If required, the leaf discs are then moved to a fresh medium every 4 or 5 weeks. After the formation of sprouts, these are cut away from the leaf discs and transferred to an identical medium.

The condition that the antisense construct has been transferred to the leaf discs is first checked by analysing leaf extracts from the regenerated sprouts in respect of glucuronidase activity by means of the substrates described by Jefferson et al (1987). The activity is demonstrated by visual assessment.

Further tests of the expression of the antisense constructs and the transfer thereof to the potato genome are carried out by southern and northern hybridisation according to Maniatis et al (1981). The number of copies of the antisense constructs that has been transferred is determined by southern hybridisation.

When it has been established that the antisense constructs have been transferred to and expressed in the potato genome, the testing of the expression on protein level begins. The testing is carried out on microtubers which have been induced in vitro on the transformed sprouts, thereby avoiding the necessity of waiting for the development of a complete potato plant with potato tubers.

Stem pieces of the potato sprouts are cut off at the nodes and placed on a modified MS medium. There they form microtubers after 2-3 weeks in incubation in darkness at 19°C (Bourque et al, 1987). The medium is composed as follows:

MS + 6% saccharose

2.5 mg/l	kinetin
2.5 mg/l	"Gellan"

The effect of the antisense constructs on the function of the GBSS gene in respect of the activity of the GBSS protein is analysed by means of electrophoresis on polyacrylamide gel (Hovenkamp-Hermelink et al, 1987). Starch is extracted from the microtubers and analysed regarding the presence of the GBSS protein. In a polyacrylamide gel, the GBSS protein forms a distinct band at 60 kD, when the GBSS gene functions. If the GBSS gene is not expressed, i.e. when the antisense GBSS gene is fully expressed so that the formation of GBSS protein is inhibited, no 60 kD band can be seen on the gel.

The composition of the starch, i.e. the proportion of amylose to amylopectin, is determined by a spectrophotometric method according to Hovenkamp-Hermelink et al (1988), the content of each starch component being determined on the basis of a standard graph.

Example 2

Extraction of amylopectin from amylopectin potato.

5 Potato whose main starch component is amylopectin, below called amylopectin potato, modified in a genetically engineered manner according to the invention, is grated, thereby releasing the starch from the cell walls.

The cell walls (fibres) are separated from fruit juice and starch in centrifugal screens (centrisiler). The fruit juice is separated from the starch in two steps, viz. first in hydrocyclones and subsequently in specially designed band-type vacuum filters.

10 Then a finishing refining is carried out in hydrocyclones in which the remainder of the fruit juice and fibres are separated.

The product is dried in two steps, first by predrying on a vacuum filter and subsequently by final drying in a hot-air current.

15 Example 3

Chemical derivatisation of amylopectin

20 Amylopectin is sludged in water to a concentration of 20-50%. The pH is adjusted to 10.0-12.0 and a quaternary ammonium compound is added in such a quantity that the end product obtains a degree of substitution of 0.004-0.2. The reaction temperature is set at 20-45°C. When the reaction is completed, the pH is adjusted to 4-8, whereupon the product is washed and dried. In this manner the cationic starch derivative 2-hydroxy-3-trimethyl ammonium propyl ether is obtained.

25 Example 4

Chemical derivatisation of amylopectin

30 Amylopectin is sludged in water to a water content of 10-25% by weight. The pH is adjusted to 10.0-12.0, and a quaternary ammonium compound is added in such a quantity that the end product obtains a degree of substitution of 0.004-0.2. The reaction temperature is set at 20-45°C. When the reaction is completed, the pH is adjusted to 4-8. The end product is 2-hydroxy-3-trimethyl ammonium propyl ether.

Example 5

35 Chemical derivatisation of amylopectin

40 Amylopectin is sludged in water to a concentration of 20-50% by weight. The pH is adjusted to 5.0-12.0, and sodium hypochlorite is added so that the end product obtains the desired viscosity. The reaction temperature is set at 20-45°C. When the reaction is completed, the pH is adjusted to 4-8, whereupon the end product is washed and dried. In this manner, oxidised starch is obtained.

Example 6

45 Physical derivatisation of amylopectin

Amylopectin is sludged in water to a concentration of 20-50% by weight, whereupon the sludge is applied to a heated cylinder where it is dried to a film.

50 Example 7

Chemical and physical derivatisation of amylopectin

55 Amylopectin is treated according to the process described in one of Examples 3-5 for chemical modification and is then further treated according to Example 6 for physical derivatisation.

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SEQ ID No. 1

Sequenced molecule: genomic DNA

Name: GBSS gene fragment from potato

Length of sequence: 342 bp

5  
 10  
 15  
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TGCATGTTTC CCTACATTCT ATTTAGAATC GTGTTGTGGT GTATAAACGT      50
TGTTTCATAT CTCATCTCAT CTATTCTGAT TTTGATTCTC TTGCCTACTG      100
TAATCGGTGA TAAATGTGAA TGCTTCCTTT CTTCTCAGAA ATCAATTTCCT      150
GTTTTGTTTT TGTTTCATCTG TAGCTTATTC TCTGGTAGAT TCCCCTTTTT      200
GTAGACCACA CATCAC ATG GCA AGC ATC ACA GCT TCA CAC CAC      243
                Met Ala Ser Ile Thr Ala Ser His His
                1                    5

TTT GTG TCA AGA AGC CAA ACT TCA CTA GAC ACC AAA TCA ACC      285
Phe Val Ser Arg Ser Gln Thr Ser Leu Asp Thr Lys Ser Thr
  10                15                20

TTG TCA CAG ATA GGA CTC AGG AAC CAT ACT CTG ACT CAC AAT      327
Leu Ser Gln Ile Gly Leu Arg Asn His Thr Leu Thr His Asn
  25                30                35

GGT TTA AGG GCT GTT      342
Gly Leu Arg Ala Val
  40
  
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SEQ ID No. 2

Sequenced molecule: genomic DNA

Name: GBSS gene fragment from potato

Length of sequence: 2549 bp

5	AAC AAG CTT GAT GGG CTC CAA TCA ACA ACT AAT ACT AAG GTA	42
10	Asn Lys Leu Asp Gly Leu Gln Ser Thr Thr Asn Thr Lys Val	
	45 50 55	
	ACA CCC AAG ATG GCA TCC AGA ACT GAG ACC AAG AGA CCT GGA	84
	Thr Pro Lys Met Ala Ser Arg Thr Glu Thr Lys Arg Pro Gly	
	60 65 70	
15	TGC TCA GCT ACC ATT GTT TGT GGA AAG GGA ATG AAC TTG ATC	126
	Cys Ser Ala Thr Ile Val Cys Gly Lys Gly Met Asn Leu Ile	
	75 80	
20	TTT GTG GGT ACT GAG GTT GGT CCT TGG AGC AAA ACT GGT GGA	168
	Phe Val Gly Thr Glu Val Gly Pro Trp Ser Lys Thr Gly Gly	
	85 90 95	
	CTA GGT GAT GTT CTT GGT GGA CTA CCA CCA GCC CTT GCA	207
	Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Leu Ala	
25	100 105 110	
	GTAAGTCCTT CTTTCATTTG GTTACCTACT CATTTCATTAC TTATTTTGTT	257
	TAGTTAGTTT CTTACTGCATC AGTCTTTTTA TCATTTAG GCC CGC GGA	304
	Ala Arg Gly	
30	CAT CGG GTA ATG ACA ATA TCC CCC CGT TAT GAC CAA TAC AAA	346
	His Arg Val Met Thr Ile Ser Pro Arg Tyr Asp Gln Tyr Lys	
	115 120 125	
35	GAT GCT TGG GAT ACT GGC GTT GCG GTT GAG GTACATCTTC	386
	Asp Ala Trp Asp Thr Gly Val Ala Val Glu	
	130 135	
40	CTATATTGAT ACGGTACAAT ATTGTTCTCT TACATTTCCCT GATTCAAGAA	436
	TGTGATCATC TGCAG GTC AAA GTT GGA GAC AGC ATT GAA ATT GTT	481
	Val Lys Val Gly Asp Ser Ile Glu Ile Val	
	140 145	
45	CGT TTC TTT CAC TGC TAT AAA CGT GGG GTT GAT CGT GTT TTT	523
	Arg Phe Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe	
	150 155 160	
50	GTT GAC CAC CCA ATG TTC TTG GAG AAA GTAAGCATAT	560
	Val Asp His Pro Met Phe Leu Glu Lys	
	165 170	

55

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TATGATTATG AATCCGTCCT GAGGGATACG CAGAACAGGT CATTTTGAGT 610  
 ATCTTTTAAC TCTACTGGTG CTTTACTCT TTTAAG GTT TGG GGC AAA 658  
 Val Trp Gly Lys 175  
 5  
 ACT GGT TCA AAA ATC TAT GGC CCC AAA GCT GGA CTA GAT TAT 700  
 Thr Gly Ser Lys Ile Tyr Gly Pro Lys Ala Gly Leu Asp Tyr 180 185  
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 CTG GAC AAT GAA CTT AGG TTC AGC TTG TTG TGT CAA 736  
 Leu Asp Asn Glu Leu Arg Phe Ser Leu Leu Cys Gln 190 195 200  
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 AATCGTTTTT TTAACCTTGT TTTCTCAG GCA GCC CTA GAG GCA CCT 832  
 Ala Ala Leu Glu Ala Pro 205  
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 AAA GTT TTG AAT TTG AAC AGT AGC AAC TAC TTC TCA GGA CCA 874  
 Lys Val Leu Asn Leu Asn Ser Ser Asn Tyr Phe Ser Gly Pro 210 215 220  
 TAT G GTAATTAACA CATCCTAGTT TCAGAAACT CCTTACTATA 918  
 Tyr G  
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 TCATTGTAGG TAATCATCTT TATTTTGCCT ATTCCTGCAG GA GAG GAT 966  
 ly Glu Asp 225  
 30  
 GTT CTC TTC ATT GCC AAT GAT TGG CAC ACA GCT CTC ATT CCT 1008  
 Val Leu Phe Ile Ala Asn Asp Trp His Thr Ala Leu Ile Pro 230 235  
 35  
 TGC TAC TTG AAG TCA ATG TAC CAG TCC AGA GGA ATC TAC TTG 1050  
 Cys Tyr Leu Lys Ser Met Tyr Gln Ser Arg Gly Ile Tyr Leu 240 245 250  
 AAT GCC AAG GTAAAATTTT TTTGTATTCA CTCGATTGCA 1089  
 Asn Ala Lys 255  
 40  
 CGTTACCCTG CAAATCAGTA AGGTTGTATT AATATATGAT AAATTTTACA 1139  
 TTGCCTCCAG GTT GCT TTC TGC ATC CAT AAC ATT GCC TAC CAA 1182  
 Val Ala Phe Cys Ile His Asn Ile Ala Tyr Gln 260 265  
 45  
 GGT CGA TTT TCT TTC TCT GAC TTC CCF CTT CTC AAT CTT CCT 1224  
 Gly Arg Phe Ser Phe Ser Asp Phe Pro Leu Leu Asn Leu Pro 270 275 280  
 50  
 GAT GAA TTC AGG GGT TCT TTT GAT TTC ATT GAT GGG TAT 1263  
 Asp Glu Phe Arg Gly Ser Phe Asp Phe Ile Asp Gly Tyr 285 290  
 GATTTTATG TTAATCAG ACCTCCAACT TTTGAATCTC TTTTGATGCT 1313

	AGTAAATTGA GTTTTAAAA TTTTGCAGAT ATGAG AAG CCT GTT AAG	1360
	Lys Pro Val Lys	
	295	
5	GGT AGG AAA ATC AAC TGG ATG AAG GCT GGG ATA TTA GAA TCA	1402
	Gly Arg Lys Ile Asn Trp Met Lys Ala Gly Ile Leu Glu Ser	
	300 305 310	
10	CAT AGG GTG GTT ACA GTG AGC CCA TAC TAT GCC CAA GAA CTT	1444
	His Arg Val Val Thr Val Ser Pro Tyr Tyr Ala Gln Glu Leu	
	315 320 325	
15	GTC TCT GCT GTT GAC AAG GGA GTT GAA TTG GAC AGT GTC CTT	1486
	Val Ser Ala Val Asp Lys Gly Val Glu Leu Asp Ser Val Leu	
	330 335 340	
20	CGT AAG ACT TGC ATA ACT GGG ATT GTG AAT GGC ATG GAT ACA	1528
	Arg Lys Thr Cys Ile Thr Gly Ile Val Asn Gly Met Asp Thr	
	345 350	
25	CAA GAG TGG AAC CCA GCG ACT GAC AAA TAC ACA GAT GTC AAA	1570
	Gln Glu Trp Asn Pro Ala Thr Asp Lys Tyr Thr Asp Val Lys	
	355 360 365	
30	TAC GAT ATA ACC ACT GTAAGATAAG ATTTTCCGA CTCCAGTATA	1615
	Tyr Asp Ile Thr Thr	
	370	
35	TACTAAATTA TTTTGTATGT TTATGAAATT AAAGAGTTCT TGCTAATCAA	1665
	AATCTCTATA CAG GTC ATG GAC GCA AAA CCT TTA CTA AAG GAG	1708
	Val Met Asp Ala Lys Pro Leu Leu Lys Glu	
	375 380	
40	GCT CTT CAA GCA GCA GTT GGC TTG CCT GTT GAC AAG AAG ATC	1756
	Ala Leu Gln Ala Ala Val Gly Leu Pro Val Asp Lys Lys Ile	
	385 390 395	
45	CCT TTG ATT GGC TTC ATC GGC AGA CTT GAG GAG CAG AAA GGT	1792
	Pro Leu Ile Gly Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly	
	400 405 410	
50	TCA GAT ATT CTT GTT GCT GCA ATT CAC AAG TTC ATC GGA TTG	1834
	Ser Asp Ile Leu Ala Val Ala Ile His Lys Phe Ile Gly Leu	
	415 420 425	
55	GAT GTT CAA ATT GTA GTC CTT GTAAGTACCA AATGGACTCA	1875
	Asp Val Gln Ile Val Val Leu	
	430	
60	TGGTATCTCT CTGGTTGAGT TTAATTGTGC CGAAGCTGAA ATTGACCTGC	1925
	TACTCATCTT ATGCATCAG GGA ACT GGC AAA AAG GAG TTT GAG	1963
	Gly Thr Gly Lys Lys Glu Phe Glu	
	435 440	

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	CAG GAG ATT GAA CAG CTC GAA GTG TTG TAC CCT AAC AAA GCT	2010
	Gln Glu Ile Glu Gln Leu Glu Val Leu Tyr Pro Asn Lys Ala	
	445 450	
5	AAA GGA GTG GCA AAA TTC AAT GTC CCT TTG GCT CAC ATG ATC	2052
	Lys Gly Val Ala Lys Phe Asn Val Pro Leu Ala His Met Ile	
	455 460 465	
10	ACT GCT GGT GCT GAT TTT ATG TTG GTT CCA AGC AGA TTT GAA	2094
	Thr Ala Gly Ala Asp Phe Met Leu Val Pro Ser Arg Phe Glu	
	470 475 480	
15	CCT TGT GGT CTC ATT CAG TTA CAT GCT ATG CGA TAT GGA ACA	2136
	Pro Cys Gly Leu Ile Gln Leu His Ala Met Arg Tyr Gly Thr	
	485 490 495	
20	GTAAGAACCA GAAGAGCTTG TACCTTTTTTA CTGAGTTTTT AAAAAAAGAA	2186
	TCATAAGACC TTGTTTTCCA TCTAAAGTTT AATAACCAAC TAAATGTTAC	2236
	TGCAGCAAGC TTTTCATTTT TGAAAATTGG TTATCTGATT TTAACGTAAT	2286
	CACATGTGAG TCAG GTA CCA ATC TGT GCA TCG ACT GGT GGA CTT	2330
	Val Pro Ile Cys Ala Ser Thr Gly Gly Leu	
	500 505	
25	GTT GAC ACT GTG AAA GAA GGC TAT ACT GGA TTC CAT ATG GGA	2372
	Val Asp Thr Val Lys Glu Gly Tyr Thr Gly Phe His Met Gly	
	510 515 520	
30	GCC TTC AAT GTT GAA GTATGTGATT TTACATCAAT TGTGTACTTG	2417
	Ala Phe Asn Val Glu	
	525	
35	TACATGGTCC ATTCTCGTCT TGATATACCC CTTGTTGCAT AAACATTAAAC	2467
	TTATTGCTTC TTGAATTTGG TTAG TGC GAT GTT GTT GAC CCA GCT	2512
	Cys Asp Val Val Asp Pro Ala	
	530	
40	GAT GTG CTT AAG ATA GTA ACA ACA GTT GCT AGA GCT C	2549
	Asp Val Leu Lys Ile Val Thr Thr Val Ala Arg Ala	
	535 540	

Name: GBSS gene fragment from potato

Length of sequence: 492 bp

GAG CTC TCC TGG AAG      GTAAGTGTGA ATTTGATAAT TTGCGTAGGT      45  
Glu Leu Ser Trp Lys  
565

ACTTCAGTTT GTTGTTC TCG TCAGCACTGA TGGATTCCAA CTGGTGTTCCT 95  
TGCAG GAA CCT GCC AAG AAA TGG GAG ACA TTG 127  
Glu Pro Ala Lys Lys Trp Glu Thr Leu  
570 575

CTA TTG GGC TTA GGA GCT TCT GGC AGT GAA CCC GGT GTT GAA 169  
Leu Leu Gly Leu Gly Ala Ser Gly Ser Glu Pro Gly Val Glu  
580 585 590

GGG GAA GAA ATC GCT CCA CTT GCC AAG GAA AAT GTA GCC ACT 211  
Gly Glu Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Thr  
595 600 605

CCT TAA ATGAGCTTTG GTTATCCTTG TTTCAACAAT AAGATCATTA 257  
Pro \*\*\*  
606

AGCAAACGTA	TTTACTAGCG	AACTATGTAG	AACCCTATTA	TGGGGTCTCA	307
ATCATCTACA	AAATGATTGG	TTTTTGCTGG	GGAGCAGCAG	CATATAAGGC	357
TGTAATAATCC	TGGTTAATGT	TTTTGTAGGT	AAGGGCTATT	TAAGGTGGTG	407
TGGATCAAG	TCAATAGAA	ATAGTTATTA	CTAACGTTTG	CAACTAAATA	457
CTTAGTAATG	TAGCATAAAT	AATACTAGAA	CTAGT		492

SEQ ID No. 4

Sequenced molecule: genomic DNA

Name: Promoter for the GBSS gene from potato

Length of sequence: 987 bp

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AAGCTTTAAC	GAGATAGAAA	ATTATGTTAC	TCCGTTTTGT	TCATTACTTA	50
ACAAATGCAA	CAGTATCTTG	TACCAAATCC	TTTCTCTCTT	TTCAAACTTT	100
TCTATTTGGC	TGTTGACGGA	GTAATCAGGA	TACAAACCAC	AAGTATTTAA	150
TTGACTCCTC	CGCCAGATAT	TATGATTTAT	GAATCCTCGA	AAAGCCTATC	200
CATTAAGTCC	TCATCTATGG	ATATACTTGA	CAGTATCTTC	CTGTTTGGGT	250
ATTTTTTTTT	CCTGCCAAGT	GGAACGGAGA	CATGTTATGA	TGTATACGGG	300
AAGCTCGTTA	AAAAAAAAATA	CAATAGGAAG	AAATGTAACA	AACATTGAAT	350
GTTGTTTTTA	ACCATCCTTC	CTTTAGCAGT	GTATCAATTT	TGTAATAGAA	400
CCATGCATCT	CAATCTTAAT	ACTAAAATGC	AACCTAATAT	AGGCTAAACC	450
AAGATAAAGT	AATGTATTCA	ACCTTTAGAA	TTGTGCATTC	ATAATTAGAT	500
CTTGTTTGTC	GTAAAAAATT	AGAAAAATATA	TTTACAGTAA	TTTGGAATAC	550
AAAGCTAAGG	GGGAAGTAAC	TAATATTCTA	GTGGAGGGAG	GGACCAGTAC	600
CAGTACCTAG	ATATTATTTT	TAATTACTAT	AATAATAATT	TAATTAACAC	650
GAGACATAGG	AATGTCAAGT	GGTAGCGTAG	GAGGGAGTTG	GTTTAGTTTT	700
TTAGATACTA	GGAGACAGAA	CCGGACGGCC	CATTGCAAGG	CCAAGTTGAA	750
GTCCAGCCGT	GAATCAACAA	AGAGAGGGCC	CATAATACTG	TCGATGAGCA	800
TTTCCCTATA	ATACAGTGTC	CACAGTTGCC	TTCTGCTAAG	GGATAGCCAC	850
CCGCTATTCF	CTTGACACGT	GTCACTGAAA	CCTGCTACAA	ATAAGGCAGG	900
CACCTCCTCA	TTCTCACTCA	CTCACTCACA	CAGCTCAACA	AGTGGTAACT	950
TTTACTCAGC	TCCTCCAATT	ATTTCTGATT	TCATGCA		987



## EP 0 788 735 A1

## SEQ ID No. 5

Sequenced molecule: genomic DNA

Name: GBSS gene from potato

Length of sequence: 4964 bp

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10	TCTATTTGGC	TGTTGACGGA	GTAATCAGGA	TACAAACCAC	AAGTATTTAA	150
	TTGACTCCTC	CGCCAGATAT	TATGATTTAT	GAATCCTCGA	AAAGCCTATC	200
	CATTAAGTCC	TCATCTATGG	ATATACTTGA	CAGTATCTTC	CTGTTTGGGT	250
	ATTTTTTTTT	CCTGCCAAGT	GGAACGGAGA	CATGTTATGA	TGTATACGGG	300
	AAGCTCGTTA	AAAAAAAAATA	CAATAGGAAG	AAATGTAACA	AACATTGAAT	350
	GTTGTTTTTA	ACCATCCTTC	CTTTAGCAGT	GTATCAATTT	TGTAATAGAA	400
15	CCATGCATCT	CAATCTTAAT	ACTAAAATGC	AACCTTAATAT	AGGCTAAACC	450
	AAGATAAAGT	AATGTATTCA	ACCTTTAGAA	TTGTGCATTC	ATAATTAGAT	500
	CTTGTTTGTC	GTAAAAAATT	AGAAAAATATA	TTTACAGTAA	TTTGGGAATAC	550
	AAAGCTAAGG	GGGAAGTAAC	TAATATTCTA	GTGGAGGGAG	GGACCAGTAC	600
	CAGTACCTAG	ATATTATTTT	TAATTACTAT	AATAATAATT	TAATTAACAC	650
	GAGACATAGG	AATGTCAAGT	GGTAGCGTAG	GAGGGAGTTG	GTTTAGTTTT	700
20	TTAGATACTA	GGAGACAGAA	CCGGACGGCC	CATTGCAAGG	CCAAGTTGAA	750
	GTCCAGCCGT	GAATCAACAA	AGAGAGGGCC	CATAATACTG	TCGATGAGCA	800
	TTTCCCTATA	ATACAGTGTC	CACAGTTGCC	TTCTGCTAAG	GGATAGCCAC	850
	CCGCTATTCT	CTTGACACGT	GTCAC TGAAA	CCTGCTACAA	ATAAGGCAGG	900
	CACCTCCTCA	TTCTCACTCA	CTCACTCACA	CAGCTCAACA	AGTGGTAACT	950
25	TTTACTCATC	TCCTCCAATT	ATTTCTGATT	TCATGCATGT	TTCCCTACAT	1000
	TCTATTATGA	ATCGTGTTGT	GGTGATATAA	CGTTGTTTCA	TATCTCATCT	1050
	CATCTATTCT	GATTTTGATT	CTCTTGCCCTA	CTGTAATCGG	TGATAAATGT	1100
	GAATGCTTCC	TTTCTTCTCA	GAAATCAATT	TCTGTTTTGT	TTTTGTTCAT	1150
	CTGTAGCTTA	TTCTCTGGTA	GATTCCCCTT	TTTGTAGACC	ACACATCAC	1199
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	Met Ala Ser Ile Thr Ala Ser His His Phe Val Ser Arg Ser					
	1 5 10					
	CAA ACT TCA CTA GAC ACC AAA TCA ACC TTG TCA CAG ATA GGA					1283
	Gln Thr Ser Leu Asp Thr Lys Ser Thr Leu Ser Gln Ile Gly					
35	15 20 25					
	CTC AGG AAC CAT ACT CTG ACT CAC AAT GGT TTA AGG GCT GTT					1325
	Leu Arg Asn His Thr Leu Thr His Asn Gly Leu Arg Ala Val					
	30 35 40					
40	AAC AAG CTT GAT GGG CTC CAA TCA ACA ACT AAT ACT AAG GTA					1367
	Asn Lys Leu Asp Gly Leu Gln Ser Thr Thr Asn Thr Lys Val					
	45 50 55					
	ACA CCC AAG ATG GCA TCC AGA ACT GAG ACC AAG AGA CCT GGA					1409
45	Thr Pro Lys Met Ala Ser Arg Thr Glu Thr Lys Arg Pro Gly					
	60 65 70					
	TGC TCA GCT ACC ATT GTT TGT GGA AAG GGA ATG AAC TTG ATC					1451
	Cys Ser Ala Thr Ile Val Cys Gly Lys Gly Met Asn Leu Ile					
	75 80					
50	TTT GTG GGT ACT GAG GTT GGT CCT TGG AGC AAA ACT GGT GGA					1493
	Phe Val Gly Thr Glu Val Gly Pro Trp Ser Lys Thr Gly Gly					
	85 90 95					

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	CTA GGT GAT GTT CTT GGT GGA CTA CCA CCA GCC CTT GCA	1532
	Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Leu Ala	
	100 105 110	
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	TAGTTAGTTT CTA CTGTCATC AGTCTTTTTC TCATTTAG GCC CGC GGA	1629
	Ala Arg Gly	
10	CAT CGG GTA ATG ACA ATA TCC CCC CGT TAT GAC CAA TAC AAA	1671
	His Arg Val Met Thr Ile Ser Pro Arg Tyr Asp Gln Tyr Lys	
	115 120 125	
15	GAT GCT TGG GAT ACT GGC GTT GCG GTT GAG GTACATCTTC	1711
	Asp Ala Trp Asp Thr Gly Val Ala Val Glu	
	130 135	
20	CTATATTGAT ACGGTACAAT ATTGTTCTCT TACATTTTCCT GATTCAAGAA	1761
	TGTGATCATC TGCAG GTC AAA GTT GGA GAC AGC ATT GAA ATT GTT	1806
	Val Lys Val Gly Asp Ser Ile Glu Ile Val	
	140 145	
25	CGT TTC TTT CAC TGC TAT AAA CGT GGG GTT GAT CGT GTT TTT	1848
	Arg Phe Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe	
	150 155 160	
	GTT GAC CAC CCA ATG TTC TTG GAG AAA GTAAGCATAT	1885
	Val Asp His Pro Met Phe Leu Glu Lys	
	165 170	
30	TATGATTATG AATCCGTCCT GAGGGATACG CAGAACAGGT CATTTTGAGT	1935
	ATCTTTTAAAC TCTACTGGTG CTTTACTCT TTTAAG GTT TGG GGC AAA	1983
	Val Trp Gly Lys	
	175	
35	ACT GGT TCA AAA ATC TAT GGC CCC AAA GCT GGA CTA GAT TAT	2025
	Thr Gly Ser Lys Ile Tyr Gly Pro Lys Ala Gly Leu Asp Tyr	
	180 185	
40	CTG GAC AAT GAA CTT AGG TTC AGC TTG TTG TGT CAA	2061
	Leu Asp Asn Glu Leu Arg Phe Ser Leu Leu Cys Gln	
	190 195 200	
	GTAAGTTAGT TACTCTTGAT TTTTATGTGG CATTTTACTC TTTTGTCTTT	2111
	AATCGTTTTT TTAACCTTGT TTTCTCAG GCA GCC CTA GAG GCA CCT	2157
	Ala Ala Leu Glu Ala Pro	
	205	
50	AAA GTT TGG AAT TTG AAC AGT AGC AAC TAC TTC TCA GGA CCA	2199
	Lys Val Leu Asn Leu Asn Ser Ser Asn Tyr Phe Ser Gly Pro	
	210 215 220	

TAT G	GTAATTAACA CATCCTAGTT TCAGAAAACT CCTTACTATA	2243
Tyr G		
TCATTGTAGG TAATCATCTT TATTTTGCCT ATTCCTGCAG	GA GAG GAT	2291
	ly Glu Asp	
	225	
GTT CTC TTC ATT GCC AAT GAT TGG CAC ACA GCT CTC ATT CCT		2333
Val Leu Phe Ile Ala Asn Asp Trp His Thr Ala Leu Ile Pro		
	230 235	
TGC TAC TTG AAG TCA ATG TAC CAG TCC AGA GGA ATC TAC TTG		2375
Cys Tyr Leu Lys Ser Met Tyr Gln Ser Arg Gly Ile Tyr Leu		
240	245 250	
AAT GCC AAG	GTAAAATTTT TTTGTATTCA CTCGATTGCA	2414
Asn Ala Lys		
255		
CGTTACCCTG CAAATCAGTA AGGTTGTATT AATATATGAT AAATTTTCACA		2464
TTGCCTCCAG GTT GCT TTC TGC ATC CAT AAC ATT GCC TAC CAA		2507
Val Ala Phe Cys Ile His Asn Ile Ala Tyr Gln		
	260 265	
GGT CGA TTT TCT TTC TCT GAC TTC CCT CTT CTC AAT CTT CCT		2549
Gly Arg Phe Ser Phe Ser Asp Phe Pro Leu Leu Asn Leu Pro		
	270 275 280	
GAT GAA TTC AGG GGT TCT TTT GAT TTC ATT GAT GGG TAT		2588
Asp Glu Phe Arg Gly Ser Phe Asp Phe Ile Asp Gly Tyr		
	285 290	
GTAATTTATGC TTGAAATCAG ACCTCCAAC TTTGAAGCTC TTTTGATGCT		2638
AGTAAATTGA GTTTTTAAAA TTTTGCAGAT ATGAG AAG CCT GTT AAG		2685
	Lys Pro Val Lys	
	295	
GGT AGG AAA ATC AAC TGG ATG AAG GCT GGG ATA TTA GAA TCA		2727
Gly Arg Lys Ile Asn Trp Met Lys Ala Gly Ile Leu Glu Ser		
	300 305 310	
CAT AGG GTG GTT ACA GTG AGC CCA TAC TAT GCC CAA GAA CTT		2769
His Arg Val Val Thr Val Ser Pro Tyr Tyr Ala Gln Glu Leu		
	315 320 325	
GTC TCT GCT GTT GAC AAG GGA GTT GAA TTG GAC AGT GTC CTT		2811
Val Ser Ala Val Asp Lys Gly Val Glu Leu Asp Ser Val Leu		
	330 335 340	
CGT AAG ACT TGC ATA ACT GGG ATT GTG AAT GGC ATG GAT ACA		2853
Arg Lys Thr Cys Ile Thr Gly Ile Val Asn Gly Met Asp Thr		
	345 350	

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CAA GAG TGG AAC CCA GCG ACT GAC AAA TAC ACA GAT GTC AAA 2895  
Gln Glu Trp Asn Pro Ala Thr Asp Lys Tyr Thr Asp Val Lys  
355 360 365

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Tyr Asp Ile Thr Thr  
370

10 TACTAAATTA TTTTGTATGT TTATGAAATT AAAGAGTTCT TGCTAATCAA 2990  
AATCTCTATA CAG GTC ATG GAC GCA AAA CCT TTA CTA AAG GAG 3033  
Val Met Asp Ala Lys Pro Leu Leu Lys Glu  
375 380

15 GCT CTT CAA GCA GCA GTT GGC TTG CCT GTT GAC AAG AAG ATC 3075  
Ala Leu Gln Ala Ala Val Gly Leu Pro Val Asp Lys Lys Ile  
385 390 395

20 CCT TTG ATT GGC TTC ATC GGC AGA CTT GAG GAG CAG AAA GGT 3117  
Pro Leu Ile Gly Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly  
400 405 410

25 TCA GAT ATT CTT GTT GCT GCA ATT CAC AAG TTC ATC GGA TTG 3159  
Ser Asp Ile Leu Ala Val Ala Ile His Lys Phe Ile Gly Leu  
415 420 425

30 GAT GTT CAA ATT GTA GTC CTT GTAAGTACCA AATGGACTCA 3200  
Asp Val Gln Ile Val Val Leu  
430

35 TGGTATCTCT CTGTTGAGT TTAAGTGTGC CGAACTGAA ATTGACCTGC 3250  
TACTCATCCT ATGCATCAG GGA ACT GGC AAA AAG GAG TTT GAG 3293  
Gly Thr Gly Lys Lys Glu Phe Glu  
435 440

40 CAG GAG ATT GAA CAG CTC GAA GTG TTG TAC CCT AAC AAA GCT 3335  
Gln Glu Ile Glu Gln Leu Glu Val Leu Tyr Pro Asn Lys Ala  
445 450

45 AAA GGA GTG GCA AAA TTC AAT GTC CCT TTG GCT CAC ATG ATC 3377  
Lys Gly Val Ala Lys Phe Asn Val Pro Leu Ala His Met Ile  
455 460 465

50 ACT GCT GGT GCT GAT TTT ATG TTG GTT CCA AGC AGA TTT GAA 3419  
Thr Ala Gly Ala Asp Phe Met Leu Val Pro Ser Arg Phe Glu  
470 475 480

55 CCT TGT GGT CTC ATT CAG TTA CAT GCT ATG CGA TAT GGA ACA 3461  
Pro Cys Gly Leu Ile Gln Leu His Ala Met Arg Tyr Gly Thr  
485 490 495

GTAAGAACCA GAAGAGCTTG TACCTTTTTA CTGAGTTTTT AAAAAAGAA 3511  
TCATAAGACC TTGTTTTCCA TCTAAAGTTT AATAACCAAC TAAATGTTAC 3561  
TGCAGCAAGC TTTTCATTTT TGAAAATTGG TTATCTGATT TTAACGTAAT 3611

	CACATGTGAG TCAG GTA CCA ATC TGT GCA TCG ACT GGT GGA CTT	3655
	Val Pro Ile Cys Ala Ser Thr Gly Gly Leu	
	500 505	
5	GTT GAC ACT GTG AAA GAA GGC TAT ACT GGA TTC CAT ATG GGA	3697
	Val Asp Thr Val Lys Glu Gly Tyr Thr Gly Phe His Met Gly	
	510 515 520	
10	GCC TTC AAT GTT GAA GTATGTGATT TTACATCAAT TGTGTACTTG	3742
	Ala Phe Asn Val Glu	
	525	
15	TACATGGTCC ATTCTCGTCT TGATATACCC CTTGTTGCAT AAACATTAAC	3792
	TTATTGCTTC TTGAATTGG TTAG TGC GAT GTT GTT GAC CCA GCT	3837
	Cys Asp Val Val Asp Pro Ala	
	530	
20	GAT GTG CTT AAG ATA GTA ACA ACA GTT GCT AGA GCT CTT GCA	3879
	Asp Val Leu Lys Ile Val Thr Thr Val Ala Arg Ala Leu Ala	
	535 540 545	
25	GTC TAT GGC ACC CTC GCA TTT GCT GAG ATG ATA AAA AAT TGC	3921
	Val Tyr Gly Thr Leu Ala Phe Ala Glu Met Ile Lys Asn Cys	
	550 555 560	
30	ATG TCA GAG GAG CTC TCC TGG AAG GTAAGTGTGA ATTTGATAAT	3965
	Met Ser Glu Glu Leu Ser Trp Lys	
	565	
35	TTGCGTAGGT ACTTCAGTTT GTTGTCTCTG TCAGCACTGA TGGATTCCAA	4015
	CTGGTGTCTT TGCAG GAA CCT GCC AAG AAA TGG GAG ACA TTG	4057
	Glu Pro Ala Lys Lys Trp Glu Thr Leu	
	570 575	
40	CTA TTG GGC TTA GGA GCT TCT GGC AGT GAA CCC GGT GTT GAA	4099
	Leu Leu Gly Leu Gly Ala Ser Gly Ser Glu Pro Gly Val Glu	
	580 585 590	
45	GGG GAA GAA ATC GCT CCA CTT GCC AAG GAA AAT GTA GCC ACT	4141
	Gly Glu Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Thr	
	595 600 605	
50	CCT TAA ATGAGCTTTG GTTATCCTTG TTTCAACAAT AAGATCATTA	4187
	Pro ***	
	606	
55	AGCAAACGTA TTTACTAGCG AACTATGTAG AACCCATTATTA TGGGGTCTCA	4237
	ATCATCTACA AAATGATTGG TTTTGTCTGG GGAGCAGCAG CATATAAGGC	4287
	TGTAAATCC TGGTTAATGT TTTTGTAGGT AAGGGCTATT TAAGGTGGTG	4337
	TGGATCAAAG TCAATAGAAA ATAGTTATTA CTAACGTTTG CAACTAAATA	4387
	CTTAGTAATG TAGCATAAAT AATACTAGAA CTAGTAGCTA ATATATATGC	4437
	GTGAATTTGT TGTACCTTTT CTTGCATAAT TATTTGCAGT ACATATATAA	4487
	TGAAATTTAC CCAAGGAATC AATGTTTCTT GCTCCGTCTT CTTTGTATGA	4537
	TTTTTATCTT AATACAGAGC TAGTGTGTTA TCTTATATAT TTTGTTTAAA	4587

	AGAAGTAATC	AAATTCAAAT	TAGTTGTTTG	GTCATATGAA	AGAAGCTGCC	4637
	AGGCTAACTT	TCAGGAGATG	GCTATTGAAT	TTCAAAATGA	TTATGTGAAA	4687
	ACAATGCAAC	ATCTATGTCA	ATCAACACTT	AAATTATTGC	ATTTAGAAAAG	4737
5	ATATTTTGA	GCCCATGACA	CATTCAATTCA	TAAAGTAAGG	TAGTATGTAT	4787
	GATTGAATGG	ACTACAGCTC	AATCAAAGCA	TCTCCTTTAC	ATAACGGCAC	4837
	TGTCTCTTGT	CTACTACTCT	ATTGGTAGTA	GTAGTAGTAA	TTTTACAATC	4887
	CAAATTGAAT	AGTAATAAGA	TGCTCTCTAT	TTACTAAAGT	AGTAGTATTA	4937
	TTCTTTCGTT	ACTCTAAAGC	AACAAAA			4964

## Claims

1. Potato plant whose genome comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising
  - a) a promoter,
  - b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.
2. Potato plant according to claim 1, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
3. Potato plant whose genome comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising
  - a) a promoter,
  - b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, with the proviso that the potato plant is not a plant variety.
4. Potato plant according to claim 3, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
5. Potato plant according to any one of claims 1-4, wherein the promoter is the GBSS promoter.
6. Potato plant according to any one of claims 1-4, wherein the promoter is selected among the CaMV 35S promoter and the patatin I promoter.
7. Potato tubers whose genome comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising
  - a) a promoter,
  - b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.
8. Potato tubers according to claim 7, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
9. Potato tubers whose genome comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising
  - a) a promoter,
  - b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said

fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, with the proviso that the tubers are not derived from a plant variety.

- 5 10. Potato tubers according to claim 9, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
11. Potato tubers according to any one of claims 7-10, wherein the promoter is the GBSS promoter.
- 10 12. Potato tubers according to any one of claims 7-10, wherein the promoter is selected among the CaMV 35S promoter and the patatin I promoter.
13. Transgenic seeds from potato plant, whose genome comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising  
15     a) a promoter,  
      b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.
- 20 14. Transgenic seeds according to claim 13, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
- 25 15. Transgenic seeds from potato plant, which potato is not a plant variety, wherein the genome of the transgenic seeds comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising  
      a) a promoter,  
      b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said  
30 fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.
- 35 16. Transgenic seeds according to claim 15, wherein said fragment has the nucleotide sequence of SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
17. Transgenic seeds according to any one of claims 13-16, wherein the promoter is the GBSS promoter.
18. Transgenic seeds according to any one of claims 13-16, wherein the promoter is selected among the CaMV 35S promoter or the patatin I promoter.
- 40 19. Microtubers of a potato plant, whose genome comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising  
      a) a promoter,  
45     b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.
- 50 20. Microtubers according to claim 19, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.
21. Microtubers of a potato plant, which potato is not a plant variety, whereby the genome of the microtubers comprises an antisense construct for inhibiting expression of the gene for granule-bound starch synthase (GBSS) in potato comprising  
55     a) a promoter,  
      b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and fragments encoding the amino acid sequences of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.

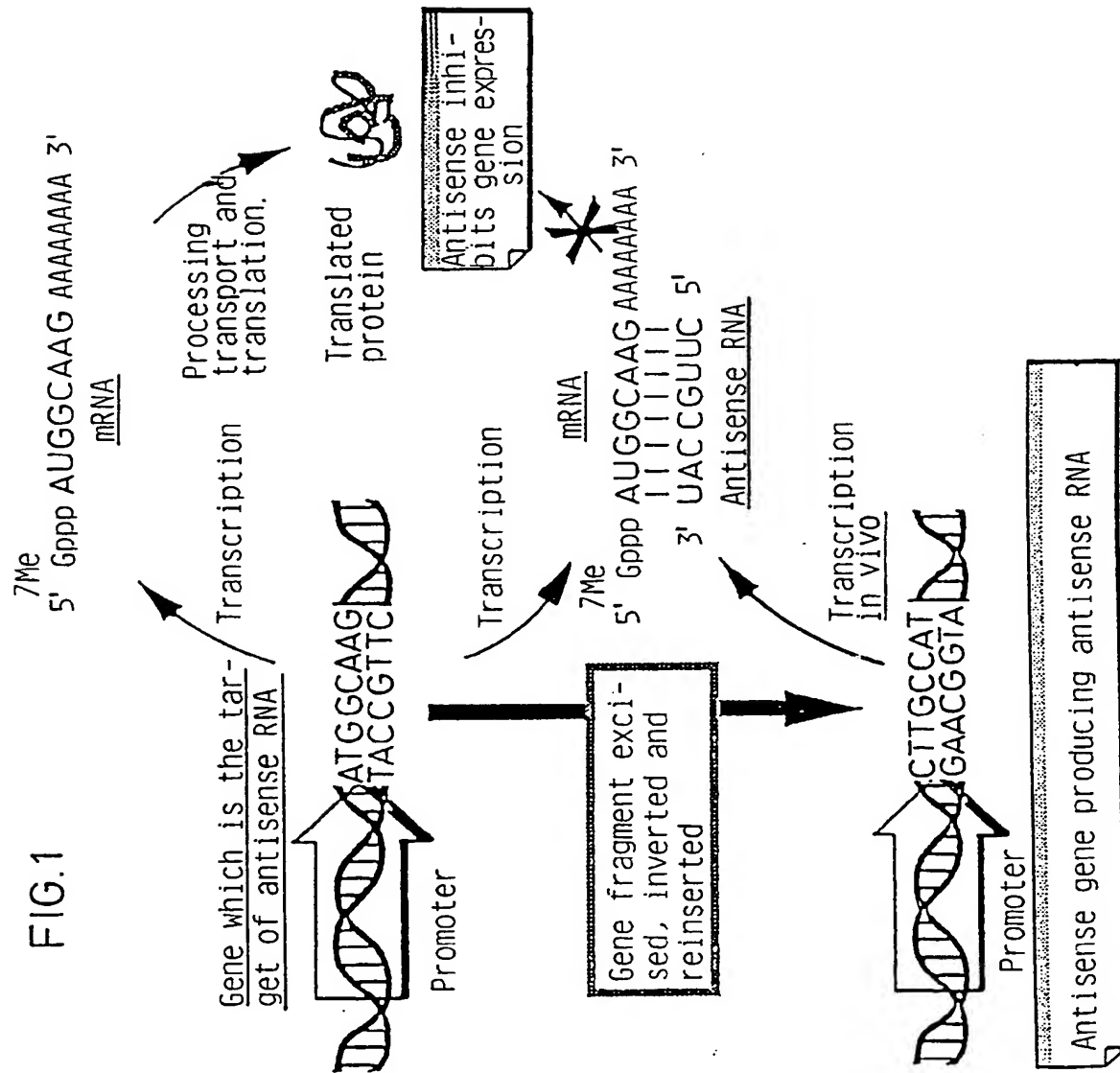
**EP 0 788 735 A1**

22. Microtubers according to claim 21, wherein said fragment has the nucleotide sequence stated in SEQ ID No. 1, or is encoding the amino acid sequence of SEQ ID No. 1.

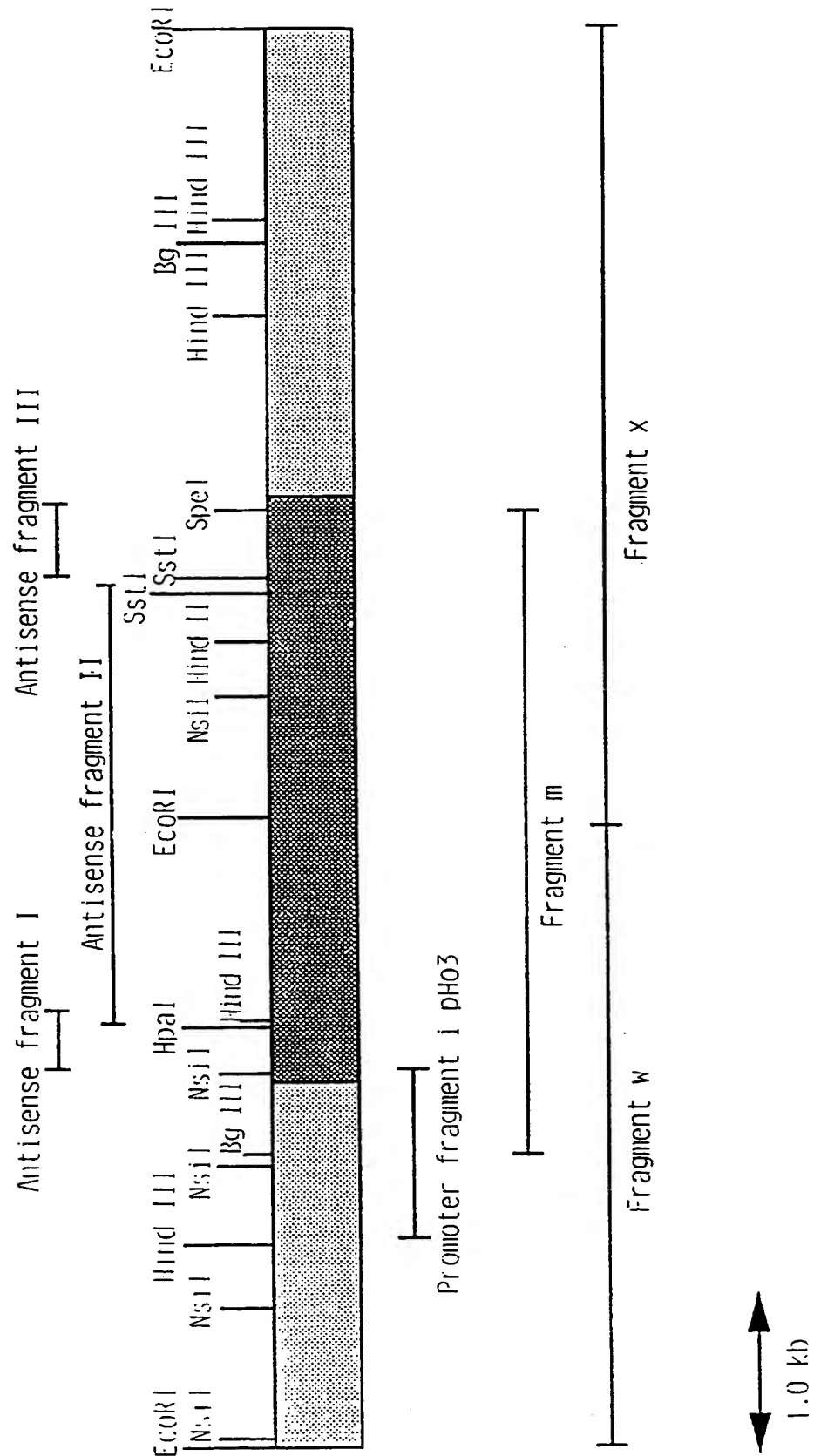
23. Microtubers according to any one of claims 19-22, wherein the promoter is the GBSS promoter.

24. Microtubers according to any one of claims 19-22, wherein the promoter is selected among the CaMV 35S promoter of the patatin I promoter.





**FIG. 2** Result of restriction analysis. GBSS coding region including introns are marked in a darker tone.



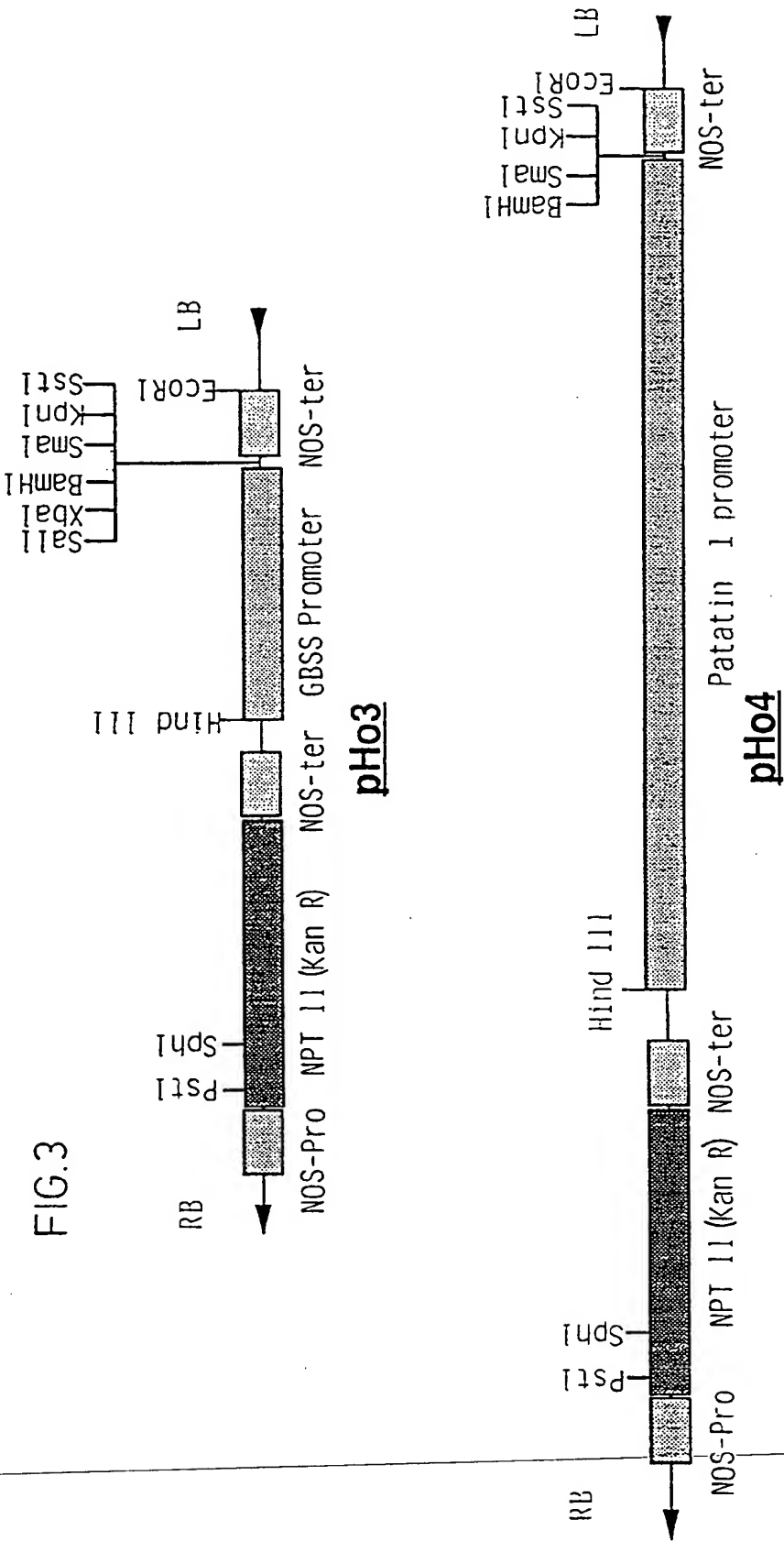


FIG. 4

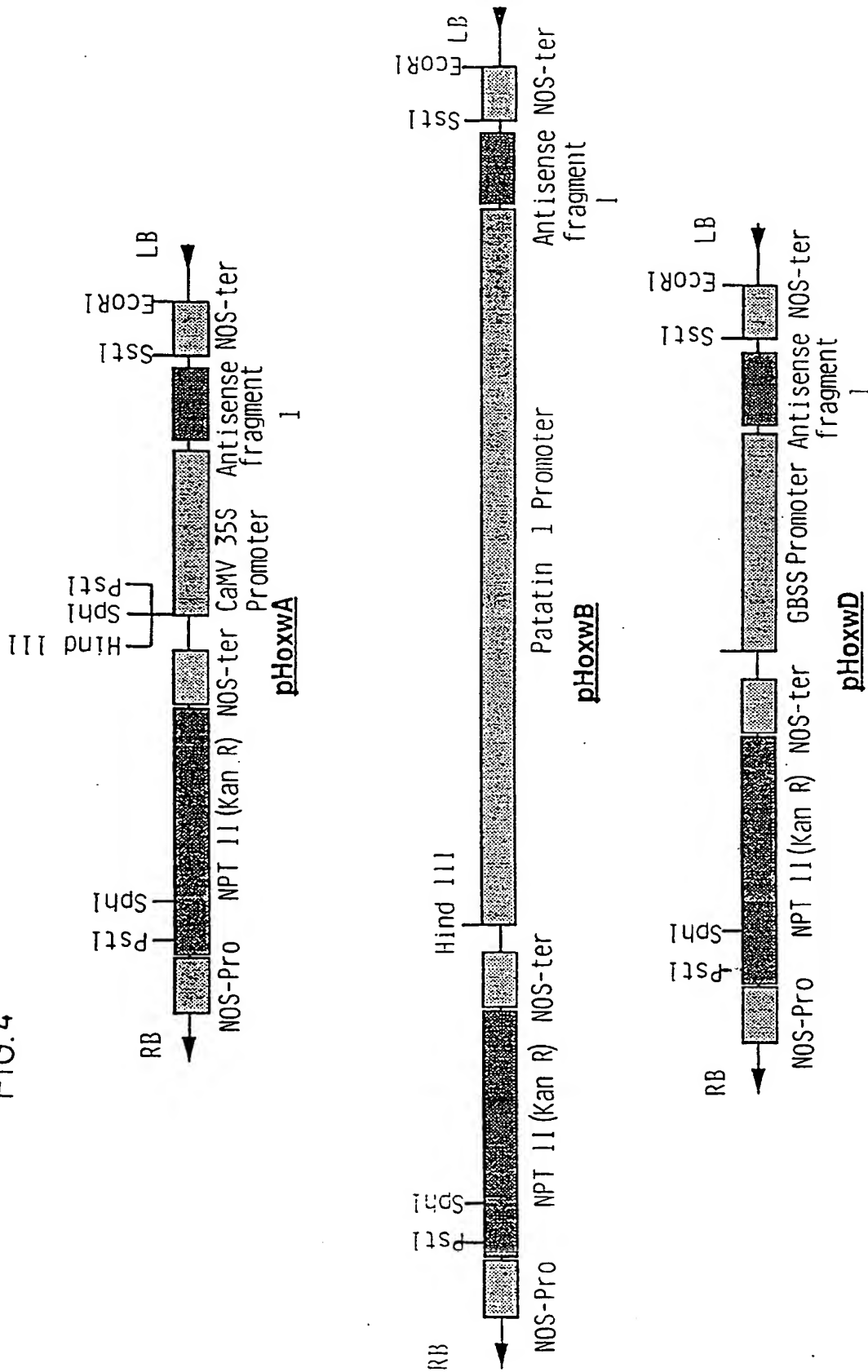


FIG. 5

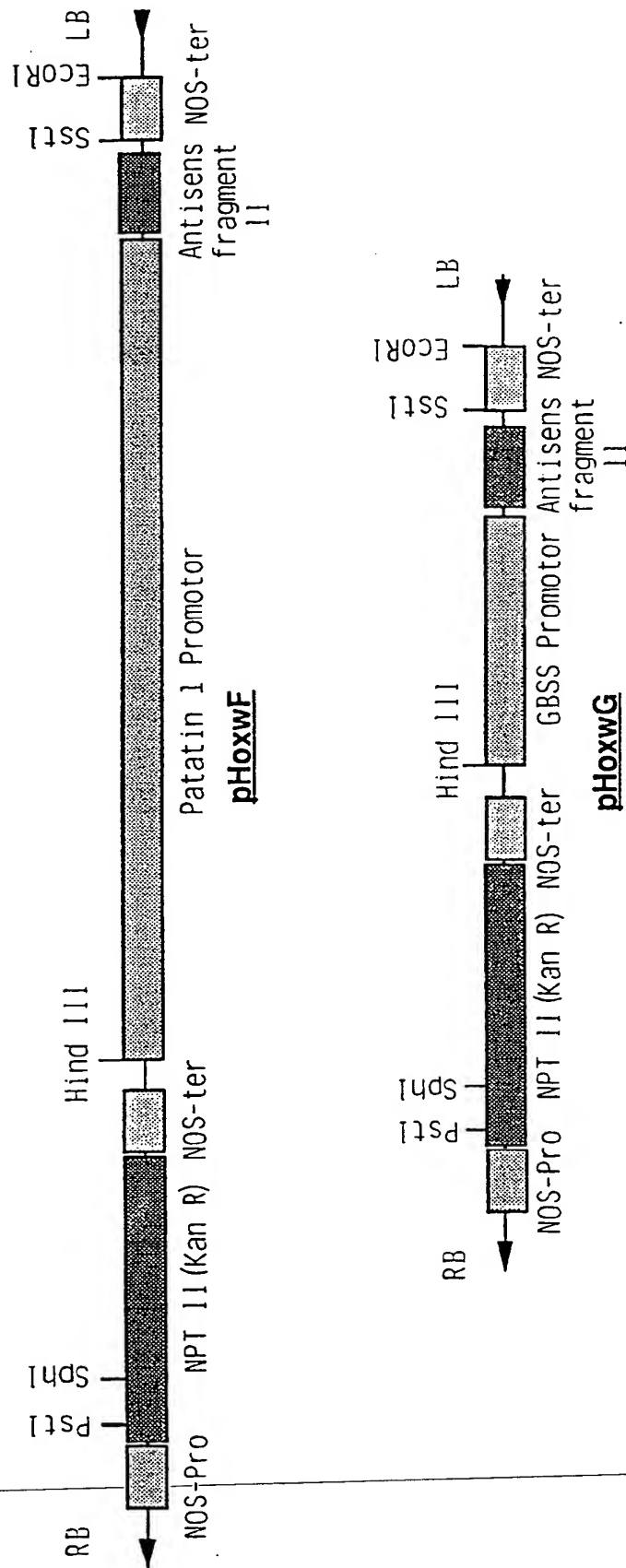
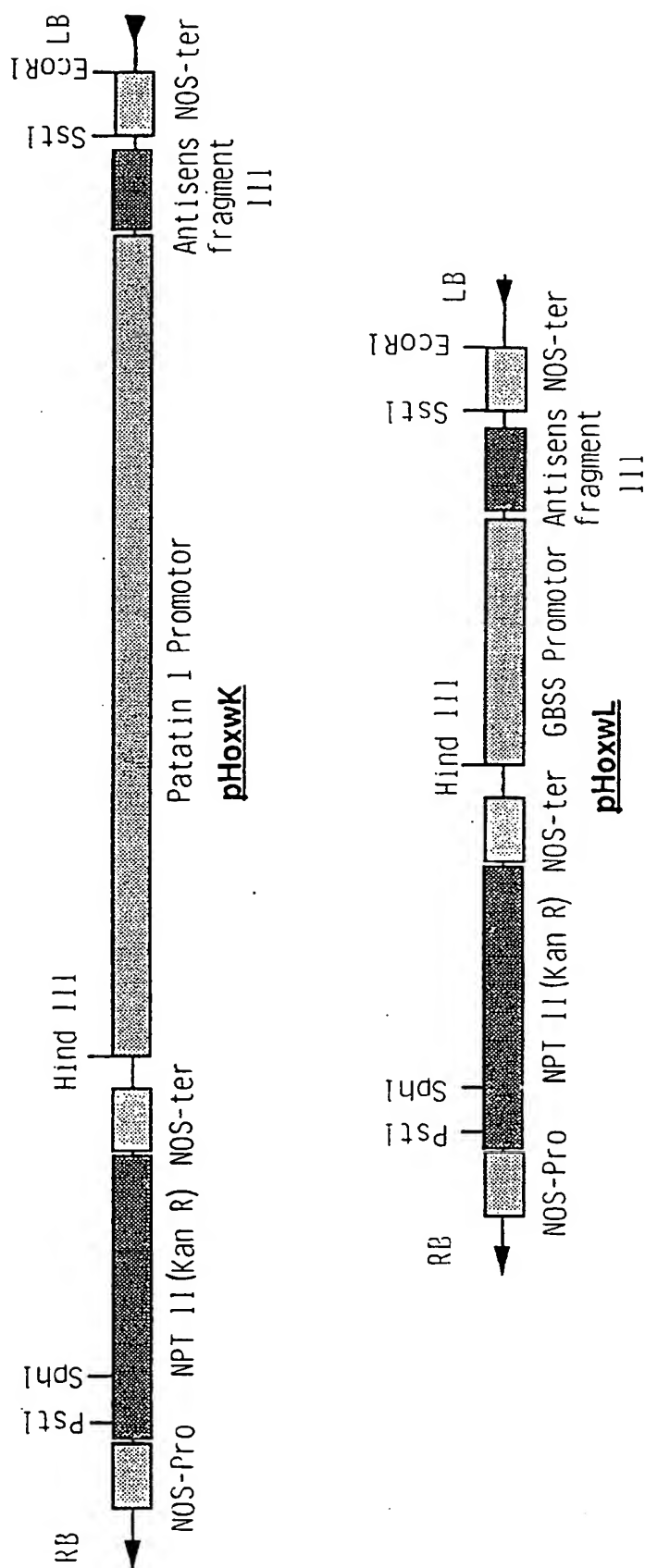


FIG.6





European Patent  
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## EUROPEAN SEARCH REPORT

Application Number  
EP 97 20 0750.4

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.6)
P,X	Mol Gen Genet, Volume 225, 1991, R.G.F. Visser et al, "Inhibition of the expression of the gene for granule-bound starchsynthase in potato by antisense constructs" page 289 - page 296 *	1-24	A01H 5/00 C12N 15/82 C12N 9/42
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A	EP 0368506 A2 (IMPERIAL CHEMICAL INDUSTRIES PLC), 16 May 1990 (16.05.90) * see claim 14 *	1-24	
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A	Plant Science, Volume 64, 1989, R.G.F. Visser et al, "Molecular cloning and Partial Characterization of the Gene for Granule-Bound Starch Synthase from a Wildtype and an Anylose-Free Potato (Solanum Tuberosum L.)" page 185 - page 192 *	1-24	
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The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl.6)
			C12N A01H
Place of search		Date of completion of the search	Examiner
STOCKHOLM		6 May 1997	PATRICK ANDERSSON
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	
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